

1 DIAGNOSIS AND TREATMENT OF DEMENTIA

2 UTILIZING THROMBOSPONDIN

3
4 FIELD OF THE INVENTION

5 The present invention relates to a method for the
6 diagnosis and treatment of dementia utilizing thrombospondin;
7 particularly to a process and device for quantifying the
8 presence of thrombospondin, e.g. a central lab or point-of-
9 care immunoassay, to wit a diagnostic kit, which utilizes
10 antibodies to determine the presence of thrombospondin in
11 circulating body fluids, thereby enabling a diagnosis of
12 dementia, particularly Alzheimer's dementia. The invention
13 further relates to processes for therapeutic intervention and
14 therapeutic targets related thereto.

15
16 BACKGROUND OF THE INVENTION

17 Cognitive impairment is a serious medical issue
18 that is of increasing concern to society. It is crucial that
19 treatment strategies are developed that effectively stop or
20 reverse the declines associated with this disorder.

21 In accordance with accepted criteria, the diagnosis of
22 dementia requires the presence of multiple cognitive deficits
23 in addition to memory impairment. Early in the disease,
24 memory impairment may be the only clinical finding, and this

1 single finding would not meet the diagnostic criteria for
2 dementia. To fulfill a diagnosis of dementia, cognitive
3 impairment must be of the degree that social or occupational
4 function is reduced, with the functional impairment
5 representing a decrease in the patient's normal ability.

6 A variety of diagnostically oriented scales exist to
7 define the degree of mental status and categorize a patient's
8 condition based upon the degree of cognitive impairment.
9 Tests such as the Short Portable Mental Status Questionnaire
10 (SPMSQ), the Folstein Mini-Mental Status Examination (MMSE)
11 or the Clinical Dementia Rating scale (CDR) usually identify
12 cognitive impairment. The MMSE includes assessments of
13 orientation, memory, attention and calculation, language,
14 ability to follow commands, reading comprehension, ability to
15 write a sentence and ability to copy a drawing. It is noted
16 that education, occupation and cultural and background
17 factors may often strongly influence MMSE scores. The CDR
18 was designed to characterize subjects from normal function
19 through various stages of dementia.

20 Age-related cognitive decline is characterized by memory
21 loss without loss of other cognitive functions. A disorder
22 similar to age-related cognitive decline is described as
23 "mild cognitive disorder" in the World Health Organizations
24 ICD-10 classification (International Statistical

1 Classification of Diseases, 10th rev.). The diagnosis of
2 mild cognitive disorder can be made if the cognitive decline
3 is temporally related to cerebral or systemic disease. Age-
4 related cognitive decline represents cognitive changes that
5 are within normal limits given the person's age. Age-
6 associated cognitive decline is characterized by a decline in
7 only one of the five broad neuropsychologic domains
8 associated with dementia: memory and learning; attention and
9 concentration; thinking; language; and visuospatial
10 functioning.

11 In accordance with findings of the International
12 Psychogeriatric Association, additional criteria should be
13 met to make a diagnosis of age-related cognitive decline.
14 These criteria include the report of cognitive decline from a
15 reliable source, a gradual onset of at least six months'
16 duration and a score of more than one standard deviation
17 below the norm on standardized neuropsychologic testing such
18 as the MMSE.

19 The term "mild cognitive impairment" (MCI) describes a
20 condition that may or may not eventually lead to dementia.
21 At least one study indicates that patients with mild
22 cognitive impairment exhibited a more rapid decline in
23 cognitive function than control patients, albeit a less rapid

1 decline than patients with mild Alzheimer's disease. Mild
2 Cognitive Impairment is often characterized by mild recent
3 memory loss without dementia or significant impairment of
4 other cognitive functions to an extent that is beyond that
5 expected for age or educational background.

6 The assumption of a relationship between Mild Cognitive
7 Impairment and AD is based on physiological similarities. It
8 has been reported that MCI patients often present with
9 significant medical temporal lobe atrophy, while others have
10 high cerebrospinal fluid and/or low CSF- $\beta\beta$ amyloid ($A\beta\beta$) 42
11 concentrations, factors that are associated with the senile
12 plaques common to AD. Furthermore, it has been reported that
13 genetic similarities exist between the conditions. The
14 strongest physiologic predictor of familial AD, for example,
15 may be the presence of apolipoprotein E gene (ApoE), and the
16 E4 allele is overrepresented in both AD and MCI patients.
17 These characteristics, in combination with the fact that the
18 onset of AD is insidious and has a course that is gradually
19 progressive, has lead practitioners to believe that
20 neuropathologies exist many years before any symptoms occur.
21 If, in fact, MCI is an early sign of AD, then the accurate
22 and early evaluation and treatment of MCI individuals might
23 prevent further cognitive decline, including development of
24 Alzheimer's disease.

1 It is apparent that the definitions of and the
2 distinctions between mild cognitive disorder, age-associated
3 cognitive decline and mild cognitive impairment remain
4 extremely controversial. Nevertheless, an advisory panel to
5 the US Food and Drug Administration recently decided that
6 mild cognitive impairment, "a condition separate from
7 Alzheimer's disease," is a valid target for new drug
8 therapies, regardless of whether a particular drug also slows
9 the progression to dementia. Furthermore, the Peripheral
10 and Central Nervous System Drugs Advisory Committee has
11 stated that more than 80% of patients with mild cognitive
12 impairment develop Alzheimer's disease within 10 years at a
13 rate of 10% to 15% of patients per year. This finding will
14 no doubt lead certain medical experts to view mild cognitive
15 impairment as early Alzheimer's disease rather than a
16 distinct condition.

17 The research literature suggests that many patients with
18 MCI progress to AD. While figures vary as to the number of
19 individuals with MCI who go on to develop AD, the percentage
20 frequently seen in the literature is up to 40% in three years
21 with a diagnosis of Mild Cognitive Impairment. Thus,
22 treatment of MCI is of great interest to clinicians in that
23 it may prevent, delay or even reverse disease-associated
24 brain deterioration.

25 Alzheimer's disease, also referred to as Alzheimer's

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1 dementia or AD is a progressive neurodegenerative disorder
2 that causes memory loss and serious mental deterioration.
3 Diagnosticians have long sought a means to definitively
4 identify AD during the lifetime of demented patients, as
5 opposed to histopathological examination of brain tissue,
6 which is the only present means available for rendering an
7 ultimate diagnosis of AD. AD is the most common form of
8 dementia, accounting for more than half of all dementias and
9 affecting as many as 4 million Americans and nearly 15
10 million people worldwide. Dementia may start with slight
11 memory loss and confusion, but advances with time reaching
12 severe impairment of intellectual and social abilities. At
13 age 65, the community prevalence of AD is between 1-2%. By
14 age 75, the figure rises to 7%, and by age 85 it is 18%. The
15 prevalence of dementia in all individuals over age 65 is 8 %.
16 Of those residing in institutions, the prevalence is about
17 50%, at any age.

18 The social impact of this disease is enormous, caused by
19 the burden placed on caregivers, particularly in the latter
20 stages of the disease. The substantial economic costs are
21 largely related to supportive care and institutional
22 admission. The rapidly increasing proportion of elderly
23 people in society means that the number of individuals
24 affected with AD will grow dramatically, therefore finding an

1 early accurate diagnosis and a cure for AD is becoming an
2 issue of major importance world wide.

3 When an individual is suspected of AD, several
4 recommended tests are performed : (1) Mini Mental State
5 Examination (MMSE) (as described above), (2) Laboratory
6 tests - complete blood count, measurement of thyroid
7 stimulating hormone, serum electrolytes, serum calcium and
8 serum glucose levels, (3) Neuroimaging - most commonly used
9 is computed tomography (CT) which has a role in detecting
10 certain causes of dementia such as vascular dementia (VaD),
11 tumor, normal pressure hydrocephalus or subdural hematoma.
12 However, neuroimaging is less effective in distinguishing AD
13 or other cortical dementias from normal aging. In primary
14 care settings, some suggest that CT could be limited to
15 atypical cases, but others recommend routine scanning.
16 Magnetic resonance imaging (MRI) currently offers no
17 advantage over CT in most cases of dementia.

18 While Alzheimer's is the most common form of dementia,
19 accounting for at least 60 % of cases, diagnostic procedures
20 for determining the exact cause of dementia, among more than
21 80 different species, is difficult at best. Furthermore, the
22 currently performed tests are inadequate in differentiating
23 AD from other types of dementia.

24 In comparison to other disease areas, the field of

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1 dementia raises questions concerning the value of diagnosis,
2 since there is often no specific cure or distinctly effective
3 therapy available. While dementia related disorders, as
4 outlined above, cannot be cured at present time, there does
5 exist symptomatic treatment, e.g. drugs such as
6 acetylcholinesterase inhibitors, which offer the hope of
7 forestalling the progression of symptoms of MCI or AD and
8 improvement of cognition and behavior are now licensed by the
9 U.S. Food and Drug Administration. Other drugs are at
10 different stages of clinical trials: (1) Drugs to prevent
11 decline in AD - DESFERRIOXAMINE, ALCAR, anti-inflammatory
12 drugs, antioxidants, estrogen, (2) Neurotrophic Factors: NGF,
13 (3) Vaccine : the recent most exciting report by Schenk et
14 al. (Nature 1999;400:173-7) raises the hope of a vaccine for
15 AD.

16 The specificity of the various therapies thus require
17 sophisticated diagnostic methodologies, having a high degree
18 of sensitivity for dementia, with particular attention being
19 drawn to MCI and AD, in order to insure their success.

20 Although there are a multitude of tests available which
21 aid in the diagnosis of AD, the only true existing diagnosis
22 is made by pathologic examination of postmortem brain tissue
23 in conjunction with a clinical history of dementia. This
24 diagnosis is based on the presence in brain tissue of

1 neurofibrillary tangles and of neuritic (senile) plaques,
2 which have been correlated with clinical dementia. Neuritic
3 plaques are made up of a normally harmless protein called
4 amyloid-beta. Before neurons begin to die and symptoms
5 develop, plaque deposits form between neurons early on in the
6 disease process. The neurofibrillary tangles are
7 interneuronal aggregates composed of normal and paired
8 helical filaments and presumably consist of several different
9 proteins. The internal support structure for brain neurons
10 depends on the normal functioning of a protein called tau. In
11 Alzheimer's disease, threads of tau protein undergo
12 alterations that cause them to become twisted. The
13 neurohistopathologic identification and counting of neuritic
14 plaques and neurofibrillary tangles requires staining and
15 microscopic examination of several brain sections. However,
16 the results of this methodology can widely vary and is time-
17 consuming and labor-intensive.

18 Given the ability of both current and prospective
19 pharmacological therapies to forestall and/or reverse the
20 onset and/or progress of various cognitive disorders, such as
21 MCI, Alzheimer's dementia and the like, an early diagnosis of
22 AD will assist to better manage the care of patients. There
23 are many cases where non-AD dementia could be confused with
24 AD dementia. Such examples include small, undetected strokes

1 which temporarily interrupt blood flow to the brain.
2 Clinically depressed patients or those with Parkinson's
3 disease can also experience lapses in memory. Many older
4 people are on a variety of medications which as a side effect
5 may, alone or in conjunction, impair their ability to perform
6 cognitive tasks.

7 Thus, if diagnostic techniques for the early
8 differentiation of dementia, particularly AD could be
9 provided, physician's would achieve an enhanced ability to
10 prescribe appropriate therapeutic intervention at an early
11 stage in the pathogenesis of this disease.

12 Various biochemical markers for AD are known and
13 analytical techniques for the determination of such markers
14 have been described in the art. As used herein the term
15 "marker" "biochemical marker" or "marker protein" refers to
16 any enzyme, protein, polypeptide, peptide, isomeric form
17 thereof, immunologically detectable fragments thereof, or
18 other molecule, whose presence, absence, or variance in
19 circulating body fluids from so-called "normal" levels, are
20 indicative of dementia. Most particularly, such markers may
21 be illustrated as being released from the brain during the
22 course of dementia related changes, e.g. AD pathogenesis.
23 Such markers include, but are not limited to, any unique
24 proteins or isoforms thereof that are particularly associated

1 with the brain.

2 There are a number of different potential uses for
3 biomarkers in evaluation of dementia, and each use could
4 involve a different marker or set of markers. Such uses may
5 include, but are not limited to, the use of a marker to
6 distinguish AD or MCI from other causes of dementia;
7 distinguishing dementia from the non-pathological effects of
8 aging; monitoring the progress of the disease after clinical
9 symptoms become apparent; utilization of a surrogate to
10 monitor the efficacy of the forthcoming therapies for AD; and
11 isolating markers which have utility as risk assessment
12 factors for AD; and identifying both the earliest biological
13 changes occurring in the brain and other changes that occur
14 as the disease progresses.

15 Ideally, it would be preferable to isolate a single
16 marker to fulfill all requirements with a high degree of
17 sensitivity and specificity, however this may be an
18 unreasonable goal. Any individual marker needs to be
19 assessed by sensitivity, specificity, reliability and
20 validity for the type of clinical situation to which it is
21 meant to apply. A marker which is poor at distinguishing AD
22 from other causes of dementia, could nevertheless be an
23 excellent marker for monitoring the progression of the
24 disease process or the response to therapy.

1 With regard to diagnostic devices, the clinical
2 evaluation and use of point-of-care tests, as well as central
3 laboratory tests utilizing biological markers are valuable
4 tools for evaluating risk, monitoring disease progression and
5 guiding therapeutic interventions. The advantages which flow
6 from the use of biological markers as diagnostic tools
7 include strengthening the certainty of the clinical
8 diagnosis, distinguishing AD from other causes of dementia,
9 and quantifying the severity of the disease and rate of
10 progression. In addition, tests using biological markers
11 should be rapid, non-invasive, simple to perform and
12 inexpensive.

13 What is lacking in the art is a relatively non-invasive
14 method and device therefore effective for definitively
15 diagnosing various forms of dementia, particularly MCI and
16 Alzheimer's dementia in living patients. Additionally, a
17 definitive method of assessing the risk of developing AD is
18 greatly needed.

19
20 DESCRIPTION OF THE PRIOR ART

21 In U.S. Pat. No. 5,508,167, Roses et al. describe
22 methods for diagnosing AD involving the detection of an
23 apolipoprotein E type 4 (ApoE4) isoform or DNA encoding
24 ApoE4. The methods can use blood samples and are analyzed by

1 an immunochemical assay. The blood sample is optionally
2 combined with a reducing agent to reduce the disulfide bond
3 in cysteine residues to the corresponding reactive sulfhydryl
4 groups. Roses et al. further describes a kit for detection
5 of the ApoE4 isoform. The test is based on the differences
6 in the amino acid sequences of the three major ApoE isoforms.
7 The test is not specific for, nor is it suggestive of the use
8 of thrombospondin as a marker for dementia.

9 Generally, most scientific papers tend to focus on the
10 peptide, β -amyloid, since it is postulated to be a major
11 determinant of AD. This is supported by the observation that
12 certain forms of familial AD mutations result in the over
13 production of β -amyloid, particularly the longer form (1-42)
14 which aggregates more readily than the shorter form. Hensley
15 et al. (Proc. Natl. Acad. Sci., (1994), 91, pp3270-3274)
16 examine the neurotoxicity based on free radical generation by
17 the peptide β -amyloid in its aggregation state. Several
18 synthetic fragments of the peptide are tested for resulting
19 neurotoxicity. Based on the fact that oxygen seems to be a
20 requirement for radical generation and glutamate synthetase
21 and creatine kinase enzymes are oxidation-sensitive
22 biomarkers, the inactivation of these enzymes are utilized as
23 indicators of active attack on biological molecules by these
24 fragmented β -amyloid aggregates.

1 Buee et al, Department of Geriatrics and Adult Development,
2 Mount Sinai School of Medicine, have conducted
3 immunohistochemical localization of thrombospondin in normal
4 human brains in the hippocampus and inferior temporal cortex.
5 The distribution of thrombospondin staining in patients with
6 Alzheimer's disease was found to be comparable to control
7 subjects. However, in patients with Alzheimer's disease a
8 subset of pyramidal neurons that may be vulnerable in
9 Alzheimer's disease exhibited decreased staining. This
10 decrease in the intensity of labeling was theorized as
11 possibly targeting a neuronal population prone to early
12 degeneration. In addition, thrombospondin staining was
13 demonstrated in senile plaques in Alzheimer's disease. These
14 results suggest that thrombospondin may be involved in the
15 process of neuronal degeneration and senile plaque formation.

16
17 SUMMARY OF THE INVENTION

18 The present invention relates to a method for the
19 diagnosis of dementia, e.g. Alzheimer's dementia (AD),
20 particularly to a method for diagnosing dementia by testing
21 for the presence of thrombospondin in body fluids,
22 particularly in blood, blood products, CSF, urine, saliva and
23 the like. The invention further relates to a process for
24 quantifying the presence of thrombospondin particularly as it

1 relates to the diagnosis of Alzheimer's dementia. More
2 particularly, the invention relates to an immunoassay
3 technique which utilizes antibodies to enable the diagnosis
4 of various forms of dementia, particularly Alzheimer's
5 dementia, as evidenced by the presence of thrombospondin.

6 The present invention relates to the use of
7 thrombospondin as a marker of dementia, particularly
8 Alzheimer's dementia, methods for determining the presence of
9 thrombospondin in body fluids, and a diagnostic device, e.g.
10 an ELISA system for diagnosing, subtyping and monitoring
11 Alzheimer's disease. The invention is based on the discovery
12 that thrombospondin is released into the circulation,
13 presumably from the brain, and can be detected in body fluids
14 outside the brain in patients suffering from Alzheimer's
15 disease.

16 Monoclonal or polyclonal antibodies which recognize
17 various epitopes of thrombospondin can be used in
18 immunoassays, wherein they enter into an immunoreaction which
19 can be monitored and/or quantified to detect circulating
20 thrombospondin proteins or the various isoforms,
21 immunological fragments, etc., as herein described, which are
22 indicative of a disease state in suspected individuals.
23 Alternatively, the thrombospondin proteins themselves may be
24 used in immunoassays to detect circulating autoantibodies in

1 such individuals. The occurrence of Alzheimer's dementia is
 2 characterized by the recognition of levels of a particular
 3 biochemical marker in bodily fluid, said levels correlating
 4 to the manifestation of Alzheimer's dementia symptoms as
 5 quantified by MMSE testing.

6 As a risk assessment test, the recognition of levels of
 7 such markers which are indicative of the presence of MCI or
 8 related dementia, and which may reasonably be deemed to be a
 9 precursor to the development of Alzheimer's dementia further
 10 augments the diagnostic capability afforded to the skilled
 11 practitioner.

12 Accordingly, it is an objective of the instant invention
 13 to provide a relatively non-invasive and highly sensitive
 14 method for the definitive diagnosis of dementia, particularly
 15 MCI and Alzheimer's disease.

16 It is a further objective of the invention to provide a
 17 method which includes analysis of at least one body fluid of
 18 a patient to determine the presence of thrombospondin as an
 19 indicator of dementia, e.g. AD vs. other forms of cognitive
 20 disorders.

21 It is a still further objective of the instant invention
 22 to provide an immunoassay effective for the recognition of
 23 thrombospondin in one or more human body fluids.

24

1 It is a still further objective of the invention to
2 provide a test kit for the diagnosis of dementia, e.g. MCI
3 and AD comprising an immunoassay test, e.g. a point-of-care
4 or central lab test, which is relatively non-invasive and
5 which can be performed utilizing a sample comprising body
6 fluids, e.g. blood or any blood products, CSF, urine, saliva
7 and the like.

8 Other objects and advantages of this invention will
9 become apparent from the following description taken in
10 conjunction with the accompanying figures wherein are set
11 forth, by way of illustration and example, certain
12 embodiments of this invention. The figures constitute a part
13 of this specification and include exemplary embodiments of
14 the present invention and illustrate various objects and
15 features thereof.

16
17 BRIEF DESCRIPTION OF THE FIGURES

18 Figure 1 is an analysis of thrombospondin levels in human
19 sera enriched by heparin affinity column;

20 Figure 2 is a Western blot analysis of thrombospondin levels
21 in human sera of Alzheimer's patients and age-matched
22 control.

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24

1

2 DETAILED DESCRIPTION OF THE INVENTION

3 Recently, a group at the University of Kentucky found
4 that the process of amyloidosis can be inhibited by low
5 molecular weight heparins. It is believed that these heparin
6 molecules bind to heparin sulfate proteoglycans and slow down
7 the process of amyloid formation. Previous reports have
8 shown that highly sulfated glycosaminoglycans are present in
9 all forms of amyloid identified, these molecules are a
10 fundamental part of basement membrane structure and may
11 provide an initiation point for amyloid fibrillogenesis.

12 With this in mind, the instant inventors set out to
13 compare the various heparin-binding molecules present in both
14 AD and control sera.

15 A protocol for fractionation and enrichment of heparin
16 sulfate proteoglycans in human sera for biomarker discovery
17 using immobilized heparin beads was determined. While not
18 wishing to be bound to any particular theory, it was
19 theorized that since heparin sulfate proteoglycans were found
20 in amyloid deposits in amyloid diseases, that they may play
21 an important role in fibril formation.

22 Materials:

23 Immobilized Heparin beads (Pierce).

24 1. 20 mM potassium phosphate buffer, pH 7.4.

- 1 2. 1.5 mL centrifuge tubes.
- 2 3. A rotator
- 3 Procedure:
- 4 Dilute 25 μ L sera samples with 500 μ L of 20 mM potassium
- 5 phosphate buffer, pH 7.4. Mix and put on ice until use.
- 6 4. Pipette 50 μ L slurry of heparin beads into a 1.5 mL
- 7 tube.
- 8 5. Wash the beads once with 500 μ L water, 3 times with 500
- 9 μ L of the phosphate buffer (spin down the beads and
- 10 remove the buffer between washes).
- 11 6. Add the sample to the beads and incubate with rotating
- 12 in cold room for 30 min.
- 13 7. Spin and remove the supernatant to another tube.
- 14 8. Wash the beads at least 3 times with 500 μ L of the
- 15 binding buffer.
- 16 9. Add 30 μ L 2x samples buffer directly to the beads and
- 17 boil for 5 min.
- 18 10. Spin, analyze the supernatant by 1D gel electrophoresis.
- 19 Heparin-conjugated agarose beads were used as an affinity
- 20 column to pull down all heparin-binding molecules in AD and
- 21 age-matched control sera samples.

22 With reference to Figure 1, an analysis of

23 thrombospondin levels in human sera enriched by heparin

24 affinity column is shown. The affinity column purified

1 samples were run on a 10-20% precast tricine gel, supplier
2 Invitrogen. The samples are: lane 1, AD120; lane 2, AD121;
3 lane 3, AD182; lane 4, AD188; lane 5, ADH39; lane 6, ADH45;
4 lane 7, ADH66; lane 8, ADC002; lane 9, N00759; lane 10,
5 N00703; lane 11, N00871; lane 12, N00910; lane 13, N00911;
6 lane14, BioRad precision protein marker. (AD refers to
7 Alzheimer's disease patients, whereas N refers to age-matched
8 normal human sera). The arrow in Fig. 1 indicates a 180 kDa
9 band that shows up in all AD samples and is not visible in
10 most of the age-matched controls. This band in gel digested
11 with trypsin was sequenced by QSTAR Pulsar I (MDS Sciex) mass
12 spectrometry. Five most intensive peaks of trypsin peptide
13 were sequenced and all of these match to thrombospondin.
14 The arrow indicates the 180 kDa band that show up in all the
15 AD samples and are not visible in 11 of the 15 age-matched
16 controls. From Fig.2 it can be seen that all 13 AD samples
17 show positive, 11 of the normals show negative, 4 of the age-
18 matched controls show negative with possible dementia.

19 Now referring to Figure 2, a Western blot analysis of
20 thrombospondin levels in human sera of Alzheimer's patients
21 and age-matched control is illustrated.

22 2 μ L of serum for each sample was run on 10-20% tricine
23 gels. The gels were transferred onto nitrocellulose membrane
24 and blocked with 5% skim milk, MBST (.1% tween twenty). 1

1 μg/mL of mouse anti human TSP-1 monoclonal antibody
2 (Thrombospondin-1, Ab-11, available from Lab Vision
3 Corporation) in PBST containing 5% skim milk was used. The
4 use of various antibody fragments is also contemplated. Goat
5 anti mouse Ab conjugated with HRP was used as secondary Ab
6 (1:4000 in PBST containing 5% skim milk). The arrow
7 indicates the 180 kDa band that show up in all the AD samples
8 and are not visible in 11 of the 15 age-matched controls.
9 From Fig.2 it can be seen that all 13 AD samples show
10 positive, 11 of the normals show negative, 4 of the age-
11 matched controls show negative with possible dementia. These
12 results show that thrombospondin can be used as a marker for
13 early diagnosis of Alzheimer's disease.

14 The markers which are analyzed according to the method
15 of the invention are released into the circulation and may be
16 present in the blood or in any blood product, for example
17 plasma, serum, cytolyzed blood, e.g. by treatment with
18 hypotonic buffer or detergents and dilutions and preparations
19 thereof, and other body fluids, e.g. CSF, saliva, urine,
20 lymph, and the like. In another preferred embodiment the
21 presence of the markers in CSF may be measured.

22 Senile plaque-dense regions of the brain of patients
23 with AD represent environments of elevated oxidative stress
24 and that protein in the brain of patients with AD is more

1 oxidized than that of controls. Reactive microglia
2 extensively present with senile plaque regions have been
3 proposed as a source of oxyradicals in the brain.

4 In a further contemplated embodiment of the invention,
5 body fluid samples may be taken from a patient at one point
6 in time or at different points in time for ongoing analysis.
7 Typically, a first sample is taken from a patient upon
8 presentation with possible symptoms of dementia and analyzed
9 according to the invention. Subsequently, some period of
10 time after presentation, for example, about 3 - 6 months
11 after the first presentation, a second sample is taken and
12 analyzed according to the invention. The data can be used to
13 diagnose AD, rule out AD, or distinguish between AD and
14 non-AD dementia. By "sample" is meant a body fluid such as
15 blood, CSF, urine, saliva, and the like.

16 The presence of thrombospondin is determined using
17 antibodies specific therefor and detecting specific binding
18 of the antibody to its respective marker. Any suitable
19 direct or indirect assay method may be used, including those
20 which are commercially available to determine the level of
21 the thrombospondin measured according to the invention. The
22 assays may be competitive assays, sandwich assays, and the
23 label may be selected from the group of well-known labels
24 such as radioimmunoassay, fluorescent or chemiluminescence

1 immunoassay, or immunoPCR technology. Extensive discussion
2 of the known immunoassay techniques is not required here
3 since these are known to those of skilled in the art. See
4 Takahashi et al. (Clin Chem 1999;45(8):1307) for S100B assay.

5 Although not limited thereto, the immunoassay method
6 used in the instant examples may comprise a double antibody
7 or sandwich ELISA for measuring the level of thrombospondin
8 in the sample. According to this method, one of the
9 antibodies is a "capture" antibody which is immobilized onto
10 a solid-phase, and the other is a "detector" antibody which
11 is labeled with, for example, an enzyme. The detector
12 antibody binds to marker protein bound to the capture
13 antibody to form a sandwich structure. A marker protein
14 standard is used to prepare a standard or calibration curve
15 of absorbance vs. marker protein concentration.

16 The assay method used to measure the presence of
17 thrombospondin should exhibit sufficient sensitivity to be
18 able to measure each protein over a concentration range from
19 normal values found in healthy persons to elevated levels in
20 people evidencing disease, i.e. 2SD above normal (= cut-off)
21 and higher.

22 The assay may be carried out in various formats,
23 including a microtiter plate format which is preferred for
24 carrying out assays in a batch mode. The assays may also be

1 carried out in automated analyzers, such as those maintained
2 at central laboratories, which are well known in the art.
3 Another assay format which can be used according to the
4 invention is a rapid manual test which can be administered at
5 the point-of-care at any location. Typically, such devices
6 will provide a result which is above or below a cut-off, i.e.
7 a semiquantitative result.

8 The protein, thrombospondin, of the present invention
9 may be used in any immunoassay system known in the art
10 including, but not limited to : radioimmunoassay, enzyme-
11 linked immunosorbent assay (ELISA), "sandwich" assays,
12 precipitin reactions, gel diffusion immunodiffusion assay,
13 agglutination assay, fluorescent immunoassays, protein A or G
14 immunoassays and immunoelectrophoresis assays. According to
15 the present invention, monoclonal or polyclonal antibodies
16 produced against thrombospondin are useful in an immunoassay
17 on samples of blood or blood products such as serum, plasma
18 or the like, spinal fluid or other body fluid, e.g. saliva,
19 urine, lymph, and the like, to diagnose patients with
20 dementia, particularly AD.

21 The antibodies can be used in any type of immunoassay.
22 This includes both the two-site sandwich assay and the single
23 site immunoassay of the non-competitive type, as well as in
24 traditional competitive binding assays. Alternatively,

1 thrombospondin may be used in a suitable assay whose goal is
2 to determine the presence of thrombospondin autoantibodies.

3 Particularly preferred, for ease and simplicity of
4 detection, and its quantitative nature, is the sandwich or
5 double antibody assay of which a number of variations exist,
6 all of which are contemplated by the present invention. For
7 example, in a typical sandwich assay, unlabeled antibody is
8 immobilized on a solid phase, e.g. microtiter plate, and the
9 sample to be tested is added. After a certain period of
10 incubation to allow formation of an antibody-antigen complex,
11 a second antibody, labeled with a reporter molecule capable
12 of inducing a detectable signal, is added and incubation is
13 continued to allow sufficient time for binding with the
14 antigen at a different site, resulting with a formation of a
15 complex of antibody-antigen-labeled antibody. The presence
16 of the antigen is determined by observation of a signal which
17 may be quantitated by comparison with control samples
18 containing known amounts of antigen.

19 In summary, the inventive concept is drawn toward a
20 process for the determination of dementia, particularly MCI
21 or Alzheimer's dementia, according to the principle of
22 immunoassay, characterized in that a serum or plasma sample
23 with at least one antibody against thrombospondin and a
24 binding partner for thrombospondin or for the antibody is

1 incubated, whereby either the antibody against thrombospondin
2 or the binding partner is labeled with a determinable group,
3 the thereby formed immunological complex which contains the
4 determinable group is separated off and the determinable
5 group in the separated off or still remaining phase is
6 determined as measure for thrombospondin from the sample. The
7 process may be further characterized in that the sample with
8 an antibody against thrombospondin and a conjugate from an
9 antibody against thrombospondin and a determinable group is
10 incubated, the formed immunological complex is separated by
11 phase separation and the determinable group is determined in
12 one of the phases; alternatively, a sample with an antibody
13 against thrombospondin and a conjugate of thrombospondin and
14 a determinable group is incubated, the formed immunological
15 complex is separated off by phase separation and the
16 determinable group is determined in one of the phases.

17 In its broadest context, the invention is directed
18 toward the use of antibodies against thrombospondin or
19 autoantibodies against thrombospondin antibodies for the
20 determination of dementia, particularly MCI or Alzheimer's
21 dementia.

22 All patents and publications mentioned in this
23 specification are indicative of the levels of those skilled
24 in the art to which the invention pertains. All patents and

1 publications are herein incorporated by reference to the same
2 extent as if each individual publication was specifically and
3 individually indicated to be incorporated by reference.

4 It is to be understood that while a certain form of
5 the invention is illustrated, it is not to be limited to the
6 specific form or arrangement of parts herein described and
7 shown. It will be apparent to those skilled in the art that
8 various changes may be made without departing from the scope
9 of the invention and the invention is not to be considered
10 limited to what is shown and described in the specification
11 and drawings.

12 One skilled in the art will readily appreciate that the
13 present invention is well adapted to carry out the objects
14 and obtain the ends and advantages mentioned, as well as
15 those inherent therein. The oligonucleotides, peptides,
16 polypeptides, biologically related compounds, methods,
17 procedures and techniques described herein are presently
18 representative of the preferred embodiments, are intended to
19 be exemplary and are not intended as limitations on the
20 scope. Changes therein and other uses will occur to those
21 skilled in the art which are encompassed within the spirit of
22 the invention and are defined by the scope of the appended
23 claims. Although the invention has been described in
24 connection with specific preferred embodiments, it should be

1 understood that the invention as claimed should not be unduly
2 limited to such specific embodiments. Indeed, various
3 modifications of the described modes for carrying out the
4 invention which are obvious to those skilled in the art are
5 intended to be within the scope of the following claims.
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